Mesothelial or Endothelial?

Sir,

We were very interested to read the recent paper by Hernando *et al.*[1] This topic is especially relevant as recent clinical trials have shown endothelial cell seeding of vascular grafts can improve patency.[2] We agree that the source of these cells derived from omental fat is at present unclear. This is unsurprising as both endothelial and mesothelial cells are derived from splanchnic mesoderm. Endothelial cells (ECs) are not a single entity and there are marked differences between those derived from macro- and microvessels. Even with conventional endothelial characteristics, difficulties do arise. Hormia reported that von Willebrand Factor (vWF) expression decreases with subculturing, so that by 9th passage, it is only expressed by 50% of Human umbilical vein ECs (HUVECs). Even fibroblasts in co-culture were shown to express vWF.[3] Weibel-Palade bodies, said to be pathognomonic for ECs, are present in only 30% of cultured Huvecs[4] and they are highest in number closest to the heart and lowest in microvessels.[5]

Cells derived enzymatically from subcutaneous fat, removing the possibility of mesothelial contamination, have been shown to be endothelial by characterisation with vWF, Ulex europaeus agglutinin-1 (UEA-1), CD31 and CD34 and by transmission electron microscopy. After the use of Percoll density gradient centrifugation (commonly employed to enhance purity) there is a decrease in expression of vWF, UEA-1 and CD31 although CD34 was reported to remain expressed.[6] Where these antigens were expressed, they appeared to be in cell clusters and occasionally singly. The authors suggested that discrepancies among previous immunohistological investigations could be due to surface antigen rearrangements occurring *in vitro*. This could account for the occasional intense staining of CD34 that was encountered by Hernando *et al.*[1]

The expression of Desmin agrees with the findings of some authors, but not with Stylianou *et al.* on their characterisation of cultured mesothelium.[7] The levels of prostacyclin produced by the cells obtained by Hernando *et al.*[1] reached values similar to those produced by cultured HUVECs agree with our previously reported findings.[8] We would agree that these cells may be suitable for seeding prosthetic grafts making the question of their origin academic.

However, the properties of these different cell lines should be investigated and compared to define differences in property and function. The only way that this may be approached is by comparison of cultured cell lines at equivalent passage from pure mesothelium, micro- and macrovessels especially as expression of the various antigens may be altered by sub-culturing. Previous studies should be interpreted with caution as most have been performed on cultured cells from omentum and compared to HUVECs to determine their origin.

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Authors' Reply

We would like to thank Mr Krijgsman *et al.* for their comments on the endothelial or mesothelial origin of the cells obtained from human omentum. We agree on the need to perform in-depth studies to establish which cell markers would be most suitable to be able to definitively determine the purity of the cell strains derived from omentum. As we understand it, one of the major problems is the lack of uniformity in the methodology employed by the different authors for obtaining and separating the mesothelial and endothelial cells present in the microcirculation of omentum.

On the basis of prior observation of the estructure and components of omentum, using both light microscopy and transmission electron microscopy, we consider that a mild, superficial treatment of the tissue using a collagenase solution is sufficient for isolation of mesothelial cells, taking care that this treatment is not aggressive enough to allow extraction of endothelial cells from the microvessels. In culture, the cells obtained through this mild digestion showed morphological and ultraestructural features of polygonal cells, with abundant extracellular secretion from the very start of the culture period, an observation which contradicts the findings of other authors.[1] However, in these cultures, there is also a certain degree of cellular contamination owing mainly to a few small colonies of cells of fibroblastic appearance, finding which does agree with those reported elsewhere.[2,3]

On the other hand, the morphological and antigenic changes in the cells over the course of the different subcultures are well known and extensively described in the literature.[4] Thus, our assays are always performed using cells from the first subculture. Another problem concerns the lack of markers specific exclusively for the mesothelial cell, a fact which is responsible for the use by different research teams of a wide range of markers, among them those typically use for muscle cells,[5] in contrast with the report of Hurlimann.[6]

One of the most relevant findings of our group refers to the behavior of these cells when seeded onto PTFE prostheses. When we compare the behavior of these cells with that of endothelial cells derived from umbilical cord vein, we observe that the layer formed by each of the two strains present evident differences, mainly with respect to stability, as well as the marked secretion of collagen fibers observed in mesothelial cells extending over the PTFE mesh; a finding that is not reported for endothelial cells. With respect to its function, the mesothelial strain exhibits antithrombogenic activity[3,5,7]; however, it is necessary to know how its possible procoagulant functions would be inhibited *"in vitro"*.[8,9] This would support the use of these cells as an alternative to endothelial cells in the seeding of vascular prostheses.

Finally, we consider it possible to obtain endothelial cells from the microvessels of omentum; however, for this purpose it is necessary to unify existing criteria and search for new ones to define the basis for discriminating between endothelial and mesothelial strains isolated from omentum.

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